

Concentration of R-spondin 2 in the Follicular Fluid is Correlated with Oocyte Number and Metaphase II Oocytes in Iraqi Women Undergo ICSI

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Abstract: ***Background:** R-spondin2 is a protein expressed in the ovary at specific stages of follicular development the effect of R-spondin-2 on the follicles mimic the effect of FSH but differs in the intracellular mechanism in signal transduction cascades some studies suggested R-spondin2 agonist could provide a new treatment regimen for infertile women with low responses to traditional ovarian stimulating therapy. **Objective:** to evaluate the concentration of R-spondin2 in the follicular and serum of women who undergo ICSI and to find if there is any correlation of the concentration of R-spondin2 with oocytes number Metaphase II(MII) number. **Methods:** follicular fluid and serum were collected from 45 women age ranged 18-42 years who undergo ICSI in the day of oocyte retrieval, the number of oocytes and MII were counted, the concentration of R-spondin2 was measured using ELISA kit. **Results:** the concentration of R-spondin 2 in the follicular fluid is correlated with oocytes number and MII number and there was no difference in the SEM between pregnant and non-pregnant women. **Conclusion:** Data of the present study show that R-spondin-2 has a direct positive effect on maturation of oocytes that provide basis for future studies about the possibility of inclusion of R-spondin2 analog in treatment of FSH unresponsive patients.*

Keywords: R-spondin2, follicle development, ICSI

1. Introduction

Assisted Reproductive Techniques are methods used to achieve pregnancy by artificial or partially artificial means and used primarily for infertility treatment, Intracytoplasmic Sperm Injection (ICSI) is a procedure in which a single sperm is injected directly into an egg cytoplasm, thus bypassing the zona pellucida and the oolemma⁽¹⁾. The successful of ICSI depend on several factors some of them are the number and the quality of the collected oocytes which reflects the success of the stimulating hormonal protocol that is given to a female to achieve higher fertilization and pregnancy rates regardless of sperm characteristics⁽¹⁾. Some studies focused on the inner environment of oocytes and paracrine factors that is secreted by the oocytes itself to regulate the maturation process and these factors mostly protein in nature and can be measured in the follicular fluid⁽²⁾. R-spondin2 is one of the members of The R-spondin (Rspo) protein family which is described as a group of four distinct secreted proteins (Rspo1-4)⁽³⁾, and their ligand-type activities mimic those of the canonical wingless ligand (Wnt), resulting in transcriptional activation mediated by the b-catenin/T cell factor (TCF) signaling pathway⁽⁴⁾. R-spondin2 necessary for development of limbs, lungs, In the early embryo and hair follicles and Its highly expressed in the ovary, developed follicles and mature oocyte⁽³⁾.

Wnt ligands activates several G-protein-coupled Frizzled receptors, and this ligand receptor interaction requires the participation of low density lipoprotein-related receptors 5 and 6 (LRP5 and 6) then passes signals into a cell through cell surface receptors. Following binding to Frizzled receptors, Wnt ligands activate the canonical Wnt pathway mediated by disheveled, glycogen synthase kinase 3 (GSK3),

and -catenin, leading to transcriptional activation of T-cell factor/lymphoid enhancer factor-regulated genes (gene transcription)⁽⁵⁾.

Wnt signaling was first identified for its role in carcinogenesis, then for its function in embryonic development. The embryonic processes it controls include body axis patterning, cell fate specification, cell proliferation and cell migration, these processes are necessary for proper formation of important tissues including bone, heart and muscle⁽⁶⁾. Wnt signaling also controls tissue regeneration in adult bone marrow, skin and intestine, research found that the genes responsible for these abnormalities also influenced breast cancer development in mice⁽⁶⁾. Studies found that⁽⁷⁾: Treatment with R-spondin2 agonist promote the early follicle development in human from primary follicle to the secondary stage. The effect of R-spondin2 on follicle growth mimic the effect of FSH but in independent cellular pathway. The study demonstrate that treatment with R-spondin2 could promote the development of human early follicles in patients with FSH low responders. So this study was designed to evaluate the concentration of R-spondin2 in the follicular and serum of women who undergo ICSI and to find if there is any correlation of the concentration of R-spondin2 with oocytes number Metaphase II(MII) number

2. Subjects and Methods

A prospective study was conducted between the first of August 2015 to the third of April 2016 at Kamal Al-Samurai hospital for infertility treatment and IVF Baghdad /Iraq. Over than three hundred women were interviewed, one hundred fifty where selected because they were matching the

inclusion criteria, the others were rejected because they were match the exclusion criteria (mentioned below).

Those women were interviewed using structural questionnaire to determine the following: history examination, type of infertility, duration of infertility, presence of other diseases, renal disease, thyroid disease, PCOS. Only Forty five women aged between (18-42) years undergoing IVF/ICSI treatment were selected and chosen for the final results because of the obstacles that occurred in this prospective study which was as follows:

- Contaminated F.F with blood no=60
- Women have hyper stimulation syndrome no=10
- Patient who is their husbands did not have sperms in the testicle biopsy
- No=10
- Patients did not cooperate and refused to participate in the study no=20
- Others no= 5

Inclusion criteria: Male factor infertility, Tubal –factor infertility, Infertility with unknown origin

Exclusion criteria: Polycystic ovarian syndrome, Endometriosis, Diminished ovarian reserve, Patients with renal disease, Patients with thyroid disease (hypo, hyper).

The level of R-spondin-2 was measured and evaluated in the serum and follicular fluid (FF) obtained from all women who participate in this study in the day of oocyte pickup. Patients were enrolled in this study were received two kinds of protocols of IVF/ICSI cycle: Long protocol ,no=7 patients with Short protocol, no =38 patients . Serum and FF R-spondin2 levels were determined by using an Enzyme Linked Immunosorbent Assay (ELISA)Kit((human R-spondin-2 ELISA kit; catalog number CSB-EL02055IHU, Cusabio, China), using the device(Biotek ELISA 216360 USA).

Results were expressed as ng/ml. The standards cover a range from 0-5 ng/ml. The sensitivity is 0.039ng/ml, detecting range0.156ng/ml-10ng/ml. R-spondin-2 Standards curve is plotted between absorbance (optical density) versus the R-spondin-2 prepared set of different standard concentration and the unknown concentration of patients' blood and follicular fluid is calculated.

Data were summarized, presented and analyzed using statistical package for social sciences (SPSS) version 23. Numeric variables were expressed as mean \pm standard error (SE), while nominal variables were expressed as number and percentage. Independent sample student t-test was used to compare mean of numeric variables between any two groups.

Pearson's correlation coefficient was used to evaluate correlation between numeric variables. P-value was considered significant when it was equal or less than 0.05

3. Results

There is a significant positive correlation between the concentration of (R- spondin2) in the follicular fluid with both oocyte number ($r=0.35, p \leq 0.05$) and MII number ($r=0.570, p \leq 0.05$). No correlation were found between serum R-spondin 2 and oocytes number and MII number ($p > 0.05$). Table (1), figure (1),(2)

Table 1: Correlation between the R-spondin2 with oocyte and MII numbers

Parameter	Oocyte no		Interpretation	MII no		Interpretation
	r	p		r	p	
S. R- spondin2	0.14	0.179	Not significant	0.32	0.131	Not significant
F. R- spondin2	0.35	0.046	significant	0.57	0.048	significant

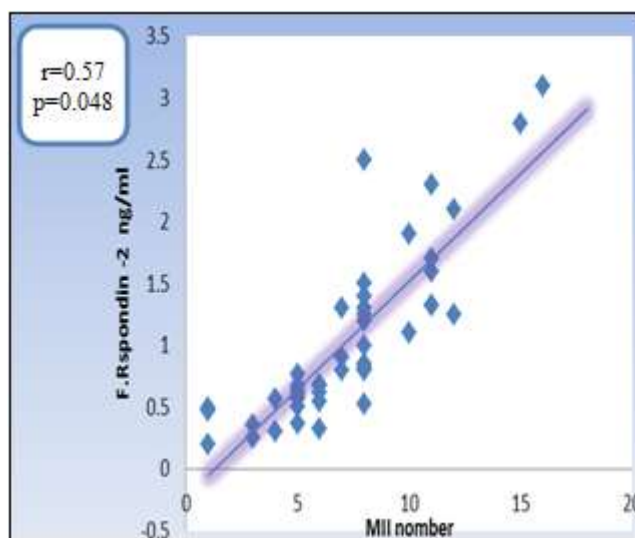


Figure 1: Correlation between F. R-spondin-2 and MII number

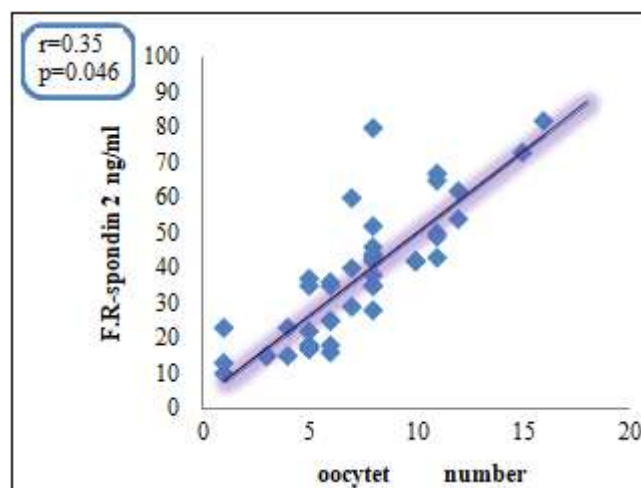


Figure 2: Correlation between F.R-spondin-2 and oocyte number

And there was no difference in the concentration of R-spondin -2 in pregnant and non pregnant women ,table(2)

Table 2: The difference in concentration of R-spondin2 in serum and follicular fluid between pregnant and non-pregnant women

Parameter	In all patients N=45 Mean ±SE	Non-pregnant N=33 Mean ±SE	Pregnant N=12 Mean ±SE	P value	Interpretation
S. Respondin-2 (ng/ml)	0.28±0.02	0.27±0.02	0.3±0.04	0.513	Not significant
F. Respondin-2 (ng/ml)	1.04±0.1	0.97±0.12	1.23±0.2	0.283	Not significant

Discussion

In contemporary IVF procedure, retrieved oocyte number is positively correlated with live birth rate. As a result of this, the success which measured live birth rate is based on obtaining enough mature follicle which contains critically well oocytes⁽⁸⁾. The ovarian follicle is the functional unit of the ovary, in which the somatic components (thecal and granulosa cells), and germ (oocyte) are closely related and interdependent⁽⁹⁾

The complete development of the oocyte within the follicular structure requires continuous two-way communication between the oocyte and cumulus cells that surround it (cumulus-oocyte complex), as well as other somatic cells included in the follicle, such as theca and the granulosa cells⁽¹⁰⁾.

The functionality and action of these cells are dependent on some factors derived from the oocyte, capable of acting directly in the coordinated processes of follicular maturation through a paracrine signaling⁽¹¹⁾. In cultured ovarian explants from prepubertal mice containing preantral follicles, treatment with R-spondin2, similar to follicle stimulating hormone, promoted the development of primary follicles to the secondary stage and *in vivo* administration of an R-spondin agonist stimulated the development of primary follicles to the antral stage in both immature mice and gonadotropin releasing hormone antagonist treated adult mice, subsequent treatment with gonadotropins allowed the generation of mature oocytes capable of undergoing early embryonic development and successful pregnancy, furthermore, R-spondin agonist treatment of immune-deficient mice grafted with human cortical fragments stimulated the development of primary follicles to the secondary stage while neutralized endogenous R-spondin2 in some ovarian explants when incubated with affinity-purified R-spondin2 antibodies lead to decreased basal ovarian weights. Thus, oocyte-derived R-spondin2 is a paracrine factor essential for primary follicle development, and R-spondin agonists could provide a new treatment regimen for infertile women with low responses to the traditional gonadotropin therapy⁽⁷⁾.

R-spondin2 and FSH likely act through independent pathways but both give the same effect by converting the immature oocyte to mature one, the only difference between them is the intracellular mechanism, while FSH start its effect when bound to its ligand receptor which is a G protein type this lead to start series in signaling transduction cascade mediated by the intracellular second messenger cAMP⁽¹²⁾ while R-spondin2 mediated its effect canonical Wnt signaling⁽¹³⁾.

Conclusion data of the present study show that R-spondin-2 has a direct positive effect on the growing and maturation of oocytes that provide basis for future studies about the possibility of inclusion of R-spondin2 analog in treatment of FSH irresponsive patients.

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